STUDIES OF MEDIA FOR THE QUANTITATIVE ESTIMATION OF BACTERIA IN WATER AND SEWAGE.*

(SECOND PAPER.†)

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IN a report from this laboratory at the Buffalo meeting the results of studies of culture media were presented, which showed that the media in general use for quantitative work enabled us to enumerate only a small percentage of the total number of bacteria in a given volume of ordinary water, and that the small percentage so obtained was not constant, but varied with different classes of water, different periods of incubation, etc. Furthermore, it was shown that higher counts were obtained by a simplification of the usual media, and that a new medium, Nährstoff agar, gave us counts many times greater than any other. The studies reported at that time were incomplete, and although much has been accomplished in the two years which have elapsed since that paper was prepared, the studies are apparently as far from completion as before. Nevertheless, certain facts have been ascertained which have an important bearing on the composition and preparation of the media, and it is thought wise to present these for discussion at this time.

The opinion has frequently been advanced that it is not good policy to destroy the existing, until the material is at hand to rebuild from the ruins a structure of greater stability. While this is perhaps true in a general sense, in scientific work it is frequently possible to pick flaws and determine errors in existing methods; and while data of this class have the tendency to destroy somewhat the confidence of the uninitiated in these methods, an impetus is given to research work which will hasten the rebuilding.

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† The first paper, under the same title, by GAGE AND PHELPS, appeared in *Jour. Am. Pub. Health Assn.*, 1901, 27, p. 393. Mr. Phelps was succeeded by Mr. Adams in January, 1903.

The data here presented are of this class—partly destructive of the present system, and at the same time serving as a groundwork for improved and more accurate methods for quantitative bacterial analyses.

A number of papers have recently appeared bearing on the different phases of the media question, but it is not the purpose of the writers to enter into a review of the literature of the subject. Two papers, however, may be mentioned in passing. Whipple* furnishes data which prove conclusively that we can never hope for any degree of accuracy or uniformity in quantitative bacteriology until we relegate gelatin media to the place to which they belong—that of confirmatory media for qualitative work or special investigations. Gustav Hesse† presents accurate data on the effect of neutralization with various alkalies and acids, on the change in reaction during sterilization, and on the effect of combinations of these two problems on the numbers of bacteria as determined by different media.

METHOD OF EXPRESSING RESULTS.

Throughout, the results of counts of bacteria are expressed as relative numbers of bacteria, these being obtained by calling the actual number on some one of the media 100, and expressing the other counts as percentages of that number. By this method of expression we have been enabled to average results obtained with waters containing widely differing numbers of bacteria, without giving undue weight to those samples which contained excessively large numbers. In nearly every case the figures given are an average of a number of separate comparisons. It is often the case that colonies on a medium become obscured, and that a lower count is obtained after the maximum is reached. In the tables we have in every case carried out this maximum as the number which would be found on each of the succeeding days, in order to eliminate, as far as possible, the error due to liquefaction or spreaders.

* *Tech. Quart.*, 1902, 15, p. 127.

† "Beiträge zur Herstellung von Nährböden und zur Bakterienzüchtung," *Ztschr. f. Hyg.*, 1903, 54, p. 1.

COMPOSITION AND PREPARATION OF MEDIA.

The standard gelatin has been of the composition and method of preparation recommended by the Bacteriological Committee. The Lawrence agar is very similar to the standard agar, but contains only 1 per cent. of agar, and varies slightly in the method of preparation. All other media, the preparation of which is not individually described, are the same as those described in the former paper.

VARIATION IN THE COMPOSITION OF BEEF-INFUSION A SOURCE OF ERROR IN CULTURE MEDIA.

In the earlier days of bacteriology it was believed that some very rich medium was necessary in order to obtain good bacterial development, and various experimenters tried different infusions, finally settling on beef as the ingredient best adapted for the purpose. The use of beef in culture media is a custom so strongly ingrained in the minds of bacteriologists at the present day that it is extremely difficult to shake their faith in this ingredient. It has often been pointed out that beef-infusion and the commercial beef extract are very variable in composition, but studies covering this variation have hitherto presented only very meager data. It has always been the custom at the Experiment Station to record the reaction of the raw beef-infusion, and of the same infusion after the albumens had been coagulated by heat and filtered out.

During 1902 determinations of solids were made on every lot of beef-infusion, both before and after the albumens were removed. Of course, this is only a very rough determination of the variation in the nutrient value of the different infusions, but, taken together with the acidity, it gives us an approximate measure of this variation. In all, fifteen lots of beef-infusion were so determined. In the raw beef-infusion we found a variation both in total and organic solids of over 2 per cent. by weight of the whole infusion. After the albumens were removed by coagulation and filtration, we found a variation of nearly 1 per cent. in these organic solids, and there was a variation in the reaction of the infusion of 1.3 per cent.

In other words, we have been attempting to make a medium

of approximately uniform composition, taking great pains that all the processes shall go on under precisely the same conditions, and that the final reaction shall be minutely adjusted to a fixed point; and we are using as a basis for that medium a substance in which the natural variation in the nutrient material is greater than the total amount of accurately determined nutrients (1 per cent. pepton) which we incorporate with it. This is, of course, assuming that the organic solids, as determined by the loss on ignition, are an approximate measure of the material in the infusion. The results of the analyses of the different lots of beef-infusion are shown in Table I:

TABLE I.

Variation in Total and Organic Solids and in Reaction of Different Lots of Beef-Infusion.

Beef-Infusion No.	Solids (Parts per 100,000)						Reaction	
	Raw			Coagulated and Filtered			Raw	Coagulated and Filtered
	Total	Organic	Fixed	Total	Organic	Fixed		
1	1,816	1,585	231	941	727	214	2.00	1.70
2	1,985	1,750	235	1,701	1,347	354	2.10	1.90
3	1,188	1,006	182	1,512	1,181	331	2.50	2.10
4	1,272	1,092	180	1,154	912	242	2.30	2.10
5	1,408	1,210	198	1,026	809	217	2.05	1.70
6	2,949	2,562	387	1,184	910	274	1.90	1.65
7	2,606	2,305	301	1,325	1,036	289	2.70	2.60
8	2,672	2,340	332	1,047	801	246	2.60	1.70
9	3,340	2,663	677	1,502	1,218	284	3.00	2.70
10	2,891	2,544	347	1,814	1,432	382	2.00	1.90
11	2,384	2,034	350	1,317	974	343	2.40	2.90
12	2,590	2,204	386	1,913	1,514	399	2.30	2.40
13	3,472	3,026	446	1,516	1,158	358	3.00	2.80
14	3,590	3,200	390	1,094	844	250	3.00	2.70
15	2,108	1,836	272	1,103	826	277	1.90	1.90
Average ..	2,418	2,091	327	1,343	1,046	297	2.38	2.18
Maximum	3,590	3,200	677	1,913	1,514	399	3.00	2.90
Minimum.	1,188	1,006	180	941	727	214	1.90	1.65

COMPARATIVE VALUE OF MERCK'S AND WITTE'S PEPTON FOR QUANTITATIVE CULTURE MEDIA.

Considerable confusion has arisen in the past through the use of various brands of pepton by different observers, and until the methods of the Bacteriological Committee recommending Witte's pepton were adopted, Merck's pepton was the one used at

Lawrence. In order to determine approximately the relative value of these two peptons, lots of pepton agar were made with each, and samples of different classes of water were plated on them, counts being made daily until the maximum count was reached, incubation being at 20° C. With four out of the five classes of water, Witte's pepton gave the higher count, and a maximum count at an earlier date than with Merck's pepton. With lake water, Merck's pepton gave better results, but these results would possibly have been changed had a larger range of surface waters been covered by the investigation, the samples being confined in this case to one source. The results of these studies are shown in Table II:

TABLE II.

Relative Numbers of Bacteria on Pepton Agar with Different Peptons.

Class of Water	Pepton	2 Days	4 Days	6 Days	8 Days	10 Days	12 Days
Sewage.....	Merck's	14	27	31	39	55	68
	Witte's	51	67	100	100	100	100
Filtered sewage.....	Merck's	4	45	65	69	69	69
	Witte's	53	69	100	100	100	100
Merrimack River ...	Merck's	16	49	62	85	93	93
	Witte's	22	39	100	100	100	100
Filtered water	Merck's	5	11	33	51	74	95
	Witte's	4	22	100	100	100	100
Lake water.....	Merck's	0	10	29	48	94	100
	Witte's	35	35	35	35	35	35
All waters	Merck's	9	33	51	67	89	98
	Witte's	38	53	100	100	100	100

Pepton agar = 1 per cent. agar, 1 per cent. pepton in water.

EFFECT OF THE KIND OF WATER USED IN MEDIA ON BACTERIAL DEVELOPMENT.

It has previously been shown that the composition of the water used in making a culture medium had considerable influence on the number of bacteria developing on the medium, and that with media made from a given water a maximum count was obtained with the same class of water. In order to study this point, five lots of plain agar were made with five different waters, as follows: regular Lawrence sewage from which the gross solids had been removed by filtration through paper; filtered sewage, *i. e.*, the

effluent from one of the sewage filters at the Experiment Station, which was giving almost complete purification; Merrimack River

TABLE III.

Chemical Analysis of Waters Used in Making Plain Agars.

(Parts per 100,000.)

Class of Water	Free Ammonia	Albuminoid Ammonia	Nitrogen As		Oxygen Consumed
			Nitrates	Nitrites	
Sewage	4.1000	0.3600	0.000	0.0000	5.20
Filtered sewage	0.1900	0.0240	4.620	0.0028	0.28
Merrimack River	0.0086	0.0164	0.011	0.0002	0.45
Filtered water	0.0006	0.0056	0.040	0.0000	0.20
Distilled water	0.0088	0.0000	0.000	0.0000	0.00

TABLE IV.

Relative Numbers of Bacteria with Different Classes of Waters on Plain Agar Made with Those Waters

Water Plated	2 Days	4 Days	6 Days	8 Days	10 Days	12 Days
Plain agar made with sewage:						
Sewage	2	40	50	87	100	100
Filtered sewage	0	0	0	0	0	0
Merrimack River	0	11	32	32	47	47
Filtered water	0	0	0	0	0	0
Plain agar made with filtered sewage:						
Sewage	7	59	59	81	81	81
Filtered sewage	0	5	5	13	16	18
Merrimack River	26	32	42	42	42	42
Filtered water	21	21	21	21	21	21
Plain agar made with Merrimack River water:						
Sewage	11	46	46	64	64	64
Filtered sewage	1	1	10	18	20	21
Merrimack River	0	21	21	47	47	47
Filtered water	0	7	14	43	57	64
Plain agar made with city water:						
Sewage	15	59	64	65	76	76
Filtered sewage	0	2	23	31	41	48
Merrimack River	16	68	74	74	74	100
Filtered water	0	14	50	93	100	100
Plain agar made with distilled water:						
Sewage	9	40	41	59	59	59
Filtered sewage	0	0	58	81	99	100
Merrimack River	16	37	42	58	58	58
Filtered water	7	14	14	14	14	21

water, Lawrence tap water, *i. e.*, Merrimack River water after filtration through a slow sand filter; distilled water, *i. e.*, the first portion distilled from a large still, this containing considerable free ammonia, but no solids. The chemical analyses of the waters used are shown in Table III.

Samples of the waters used in the preparation were saved, and plated out on the various media. With sewage the maximum count was obtained on the agar made with sewage. The maximum growth with the polluted river water and with the filtered water was obtained on the agar made with filtered water, while the filtered sewage, the purest water of all from a bacteriological standpoint, showed the best development on the agar made with distilled water. Both filtered water and filtered sewage failed to show any growth on media made with sewage. Other samples of water of the same classes followed the same general laws as to preference as did the waters with which the media were made. The relative numbers of bacteria with the same waters used in the preparation of the various media are shown in Table IV.

EFFECT OF THE SALTS PRESENT IN COMMERCIAL AGAR ON THE DEVELOPMENT OF BACTERIA.

It has been pointed out that one of the sources of irregularity in the use of agar media has probably been the variation in the natural salt content of the crude agar. In order to test this phase of the problem, a lot of commercial agar was divided in two portions, one portion of which was used to make plain agar, the other portion being allowed to soak over night in a 5 per cent. solution of glacial acetic acid* in distilled water, and then washed three to four hours in running water, after which plain agar was made as with the first portion. The theoretical effect of the acetic acid would be to decompose the compounds of organic acids with the alkaline bases, forming soluble compounds which would readily be washed out. No analysis was made to determine what proportion of the salts was removed by this process.

Different classes of water were plated on the two media, and daily counts made as usual until a maximum count was obtained.

*The treatment with acetic acid was the suggestion of Dr. George T. Moore.

The development of bacteria was slightly better on the agar from which the salts had been removed than on the commercial agar, although with two classes of waters the commercial agar gave higher numbers than the purified agar. The results of comparisons of these two media are shown as follows in Table V:

TABLE V.

Relative Numbers of Bacteria on Plain Agar Made with Commercial Shred Agar and with Washed Commercial Shred Agar.

CLASS OF WATER	COMMERCIAL AGAR				WASHED AGAR			
	2 Days	4 Days	6 Days	8 Days	2 Days	4 Days	6 Days	8 Days
Sewage	13	15	29	31	27	65	100	100
Filtered sewage	36	91	91	91	22	80	99	100
Merrimack River	42	71	97	100	17	55	94	96
Filtered water	40	40	85	100	37	38	67	73
All waters	35	57	80	85	27	63	95	100

EFFECT OF GLYCERIN.

Considerable contention has arisen in the past as to whether it was a benefit to use a glycerinated agar for bacterial counts. We know that there are certain forms of bacteria which will grow on a glycerinated medium, but will not grow on standard agar. These forms are, however, probably never met with in water analysis, and their behavior should not be considered in this connection. Until within about two years an agar containing 3 per cent. of glycerin was the regular medium used at Lawrence, the glycerin being omitted from the agar at the time the methods were changed, to agree with the recommendation of the Bacteriological Committee. The use of glycerin agar in preference to agar without glycerin was based on experiments made in the early history of the Experiment Station, which data have been accidentally destroyed. Studies have been made with agar containing various percentages of glycerin, which show that somewhat better development is obtained on media without the glycerin. With the majority of waters, however, variations in the amount of glycerin have caused only a comparatively slight variation in the relative numbers. With filtered sewage the best results were obtained on

a medium containing 6 per cent. of glycerin, the medium containing 1 per cent. of glycerin being a close second, and the medium containing no glycerin coming third. With sewage there was a maximum deviation of only 1 per cent. between media containing no glycerin and any of the media containing glycerin. With water polluted by sewage this deviation was considerably greater, while with filtered water approximately the same development occurred on the medium containing 2 per cent. of glycerin and on the medium containing no glycerin. The results of the determinations on these media are shown as follows in Table VI:

TABLE VI.

Relative Numbers of Bacteria on Beef-Pepton Agar Containing Varying Amounts of Glycerin.

Class of Water	Percentage of Glycerin						
	0	1	2	3	4	5	6
Sewage.....	100	96	98	93	95	96	93
Filtered sewage....	83	96	68	72	59	68	100
Merrimack River..	100	94	83	68	62	75	64
Filtered water.....	100	72	100	37	77	69	62
All waters.....	100	94	90	71	77	81	86

STUDIES OF NÄHRSTOFF AGAR.

In the previous paper it was shown that higher counts of bacteria were obtained with Nährstoff agar than with any other medium, and it was stated that this was probably due to the fact that certain species of bacteria which do not grow on the usual media do flourish on this medium, and that many debilitated individual bacteria which have not the vitality to produce colonies on the usual media are able to develop slowly on this medium. In order to test the truth of this last statement, experiments have been made with pure cultures of different species of bacteria. Three lines of investigation have been carried out with each. The cultures were put through three generations in broth, then transferred to agar streaks, and, after a two-day incubation, water suspensions were made of the surface growth. After being thoroughly shaken, these were plated out on Lawrence agar, standard gelatin,

and Nährstoff agar. The water suspensions were then placed on ice, and allowed to stand two days, after which they were thoroughly shaken and again plated on the three media. Water suspensions were also made from agar slants on which the growth was 30 days old, and plated as before. These three methods gave a check upon one another the first giving us as nearly as possible all active bacteria; the second, the condition where the bacteria had been subjected to an unfavorable state; and the third, a case where the majority of the individual bacteria were debilitated by the length of time they had stood on media. The cultures were selected to cover as many types of water bacteria as possible, in all eighteen cultures being studied.

The results show that our former belief that certain debilitated individuals would develop on Nährstoff agar when they would not develop on standard gelatin or agar, was not well grounded, in nearly every case the counts on the Nährstoff agar being less than on the other two media, and in the majority of cases the counts on

TABLE VII.

Relative Development of Pure Cultures of Bacteria on Standard Gelatin, Lawrence Agar, and Nährstoff Agar.

Group*	Name*	Water Suspension from Fresh Culture			Water Suspension after Two Days on Ice			Water Suspension from Thirty-Day-Old Culture		
		Gelatin	Agar	Nährstoff Agar	Gelatin	Agar	Nährstoff Agar	Gelatin	Agar	Nährstoff Agar
1129	Sewage streptococcus No. 131	100	95	1	100	100	0	100	123	0
1129	Sewage streptococcus No. 191	100	72	0	100	79	0	100	90	0
1229	Micrococcus No. 111	100	84	90	100	64	78	...	...	..
1354	Sarcina Lutea No. 26	100	145	0	100	43	2	100	53	14
2139	Bacterium No. 110	100	83	91	100	83	100	100	100	90
2139	Bacterium No. 116	100	57	21	100	44	33	100	72	19
2139	Bacterium No. 117	100	82	76	100	100	100	100	94	100
2269	Pseudomonas No. 126	100	80	75	100	100	97	100	166	230
2429	B. typhosus No. 143	100	99	42	...	...	...	...	...	..
2429	B. typhosus No. 145	100	62	20	...	...	...	...	...	..
2429	Bacillus No. 89	100	59	56	100	80	60	100	90	21
2429	B. intestinalis No. 128	100	80	96	100	86	77	100	83	50
2439	B. coli No. 192	100	38	37	100	35	22	100	46	64
2439	Bacillus No. 51	100	83	84	100	92	75	100	80	32
2439	Bacillus No. 56	100	74	75	100	37	87	100	53	0
2439	Bacillus No. 58	100	75	67	100	63	18	100	55	25
2439	Bacillus No. 107	100	92	74	100	98	59	100	50	89
2449	Bacillus D (Bact. comm.)	100	64	61	100	58	50	...	...	..
2469	Bacillus B (Bact. comm.)	100	65	29	100	28	5	100	176	62

*For descriptions of Lawrence Species and methods of grouping see *Rept. Mass. Board of Health*, 1901, p. 415.

gelatin being the highest. With the old cultures a greater relative number of bacteria developed on the Nährstoff agar than with the fresh cultures. It may be, however, that our range of cultures did not include the specific types which would cause the increased counts on the Nährstoff agar with ordinary waters.

Some attempts have also been made to prove that species naturally present in water, which would not grow on the usual gelatin or agar, were responsible for the increased counts on the Nährstoff agar. To show this, a large number of colonies from the Nährstoff agar plates have been transferred to Nährstoff agar streaks, and the growth from these have been tested to see whether the species would grow on ordinary gelatin and agar. So far, however, these results have proved very unsatisfactory, and have not given us the information we desired.

The results of the counts of the three types of cultures with the different species are shown in Table VII.

THE EFFECT OF DECREASING THE AMOUNT OF NÄHRSTOFF IN NÄHRSTOFF AGAR.

The fact that a reduction in the amount of nutrients in the usual media allowed of an increase in the bacterial counts led us to believe the same might be true with Nährstoff, and that a better growth would be obtained on media containing less than

TABLE VIII.

Relative Numbers of Bacteria on Nährstoff Agar of Different Strengths.

Class of Water	Percentage of Nährstoff	2 Days	4 Days	6 Days	8 Days	10 Days
Sewage	1.0	10	19	25	25	25
	0.5	45	59	97	100	100
Filtered sewage ..	1.0	20	35	35	43	43
	0.5	35	89	96	100	100
Merrimack River .	1.0	14	41	55	55	55
	0.5	14	47	83	100	100
Filtered water....	1.0	2	29	45	54	59
	0.5	12	88	94	100	100
Lake water.....	1.0	0	4	7	13	13
	0.5	5	81	100	100	100
All waters....	1.0	9	25	33	38	39
	0.5	22	73	94	100	100

1 per cent. of Nährstoff. Two lots of Nährstoff agar were made, one containing 1 per cent. as usual, and the other one-half this amount (0.5 per cent.); and comparative platings were made on the two media with the various classes of water. In every case an increased number of bacteria appeared on the medium which contained the smaller amount of Nährstoff. The results of these experiments are shown in Table VIII.

EFFECT OF COOKING NÄHRSTOFF AGAR IN OTHER THAN NEUTRAL SOLUTION

It is well known that better results have been obtained in the preparation of ordinary culture media if the cooking were done in a solution which was slightly alkaline, and the usual methods of preparing standard gelatin and agar have allowed for this fact. It is believed that the reason for this was the breaking down of many of the albumens, either into albumens of less complex structure or into simpler compounds. Nährstoff being a compound of albumens and albumoses, it was reasonable to believe that the method of preparing in neutral solution might not be the procedure best adapted to obtain the most favorable medium for bacterial development. Three lots of Nährstoff agar were made of the same percentage composition. All of these were allowed to cook slowly for one hour on the water bath, one of them being unchanged by the addition of acid or alkali, to one of them sufficient normal potassium hydrate being added to make the solution about 1 per cent. alkaline, and to the third sufficient normal hydrochloric acid being added to make the reaction about plus 1 per cent. After cooking, acid or alkali was added in sufficient amount to bring them all back to the neutral point, and they were then filtered, tubed, and sterilized as usual. Titrations made after sterilization showed that the three media were of the same reaction. Comparative platings were then made on the three media with waters of different classes. As had been expected, the media made in alkaline solution gave higher counts with four out of five waters. With sewage the media made in acid solution gave the highest counts. The results obtained with the different classes of water on these media are shown as follows in Table IX:

TABLE IX.

Relative Numbers of Bacteria on Nährstoff Agar of the Same Strength Made by Different Procedures.

Class of Water	Procedure	2 Days	4 Days	6 Days	8 Days	10 Days
Sewage	Neutral	25	46	62	62	62
	Alkaline	42	59	71	85	85
	Acid	47	82	100	100	100
Filtered sewage	Neutral	30	53	54	54	54
	Alkaline	44	70	89	100	100
	Acid	33	49	67	73	73
Merrimack river	Neutral	14	37	50	54	54
	Alkaline	10	68	92	100	100
	Acid	10	69	81	92	95
Filtered water	Neutral	2	31	49	59	66
	Alkaline	12	64	89	100	100
	Acid	17	42	55	58	58
Lake water	Neutral	0	4	6	11	11
	Alkaline	5	41	71	98	100
	Acid	4	59	82	82	82
All waters	Neutral	15	35	46	50	51
	Alkaline	23	62	85	100	100
	Acid	23	62	80	84	84

THE EFFECT OF VARIATION IN MEDIA ON THE DETERMINATION OF THE EFFICIENCY OF WATER FILTERS IN THE REMOVAL OF BACTERIA.

The principal use of counts of bacteria at the Experiment Station has been in the determination of the numbers of germs in the applied waters and effluents from the various water filters, and it has always been assumed that, although the different lots of media vary among themselves, the ratio between the numbers in the applied waters and in the effluents would be approximately a constant on media of the same kind; in other words, that we had comparative results. The use of agar instead of gelatin for work of this class has always been thought preferable, since it has been proved that agar was less subject to variation than gelatin. It has always been the practice at Lawrence to compare directly each lot of agar and of gelatin with the preceding lot and the agar and the gelatin, gelatin being the standard medium in so many other laboratories that our idea was to approximate the counts on the agar to those which would be obtained on the standard gelatin. In each comparison we have plated all the classes of water at the Experiment Station, and from comparisons made during 1902 we

have traced out the variation in the efficiency of some of the water filters, as determined by the counts on agar and by those on gelatin. In thirty-nine comparisons during the year an applied water for, and an effluent from, a filter were plated on the various lots of media from which we could figure the efficiency of the filter for the day. In a considerable number of comparisons the efficiency, as figured by two or more agars, proved to be the same, but in no case was it found that two lots of gelatin gave the same efficiency figure, the minimum deviation between any two lots of gelatin being 0.10. The results of the estimation of efficiency by different lots of gelatin and agar were as follows:

Average maximum deviation between different gelatins	0.92
Average maximum deviation between different agars	0.53
Maximum deviation between different gelatins	4.50
Maximum deviation between different agars	5.00
Minimum deviation between different gelatins	0.10
Minimum deviation between different agars	0.00
Average deviation of agar from gelatin	+0.09
Number of times gelatin greater than agar	16
Number of times agar greater than gelatin	18
Number of times agar and gelatin were the same	50

EFFECT OF COMPOSITION OF MEDIA AND PERIOD OF INCUBATION ON THE CALCULATED EFFICIENCY OF WATER FILTERS.

In a number of comparisons of the various media which we have studied we have plated an applied water and an effluent on the different media on the same day, and we have thus been able to calculate and compare the efficiency as determined by the various media and after different periods of incubation. In Table X four such comparisons are shown. The difference between the efficiency, as determined on various media by counts on different days, has been relatively small in many instances; still the maximum difference has been sufficient, if the variation occurred in routine work, to cause a false opinion as to the quality of the work the filter was doing.

The whole question of efficiency is based on the assumption that the ratio between the determined number of bacteria and the total number of any medium is a constant, for both applied water and effluent should be the same. If a greater percentage of the

total number of bacteria in the applied water develop on a given medium than was the case with the effluent, we should obtain an efficiency value which was too high; and, on the other hand, if the reverse be true, we should obtain an efficiency value which was too low. In Table XI are shown the relative numbers of bacteria on a raw water and the effluents from two water filters (A and B), on agar of various reactions; and also the various efficiency values for these two filters as calculated from the actual counts. The effect

TABLE X.

Variation in the Percentage Removal of Bacteria by Water Filters when Estimated by Counts on Different Media and after Different Periods of Incubation.

MEDIA	SERIES I				SERIES II			
	Two Days	Four Days	Six Days	Eight Days	Two Days	Four Days	Six Days	Eight Days
Plain agar	99.9	99.9	99.8	99.7	98.7	98.7	98.9	98.8
Pepton agar	....	....	....	....	....	....	....	....
Beef agar	99.8	99.9	99.9	99.9	98.8	98.7	98.9	98.9
Lawrence agar	99.8	99.8	99.8	99.8	98.8	99.1	99.2	99.2
Plain gelatin	....	....	....	....	....	....	....	....
Pepton gelatin	....	....	....	....	....	....	....	....
Beef gelatin	....	....	....	....	....	....	....	....
Standard gelatin	....	....	....	....	....	....	....	....
Nährstoff agar	99.7	99.0	99.0	99.0	93.8	96.8	96 8	96.8
Nährstoff pepton agar.	99.8	99.5	99.5	99.5	98.6	98.0	98.1	98.1
Nährstoff beef agar	99.9	99.5	99.3	99.4	98.4	98.7	98.7	98.7
Nährstoff glycerin agar	100.0	98.8	99.8	99.0	99.0	97.7	97.9	97.9

MEDIA	SERIES III				SERIES IV			
	Two Days	Four Days	Six Days	Eight Days	Two Days	Four Days	Six Days	Eight Days
Plain agar	95.7	95.4	95.3	95.8	99.8	99.7	99.7	99.7
Pepton agar	99.7	98.9	98.9	99.0	99.8	99.9	99.9	99.9
Beef agar	99.9	99.7	99.7	99.7	99.9	99.7	99.8	99.8
Lawrence agar	99.4	99.7	99.7	99.7	99.3	99.6	99.6	99.6
Plain gelatin	99.7	98.1	98.1	97.7	99.9	99.7	99.7	99.8
Pepton gelatin	97.5	98.3	98.3	98.3	99.8	99.8	99.8	99.8
Beef gelatin	97.0	97.4	96.4	96.5	99.8	99.5	99.6	99.6
Standard gelatin	99.4	99.1	99.5	99.5	99.4	99.5	99.6	99.6
Nährstoff agar	95.0	97.9	97.4	97.6	99.8	98.5	97.9	97.9
Nährstoff pepton agar.	....	....	....	....	....	....	....	....
Nährstoff beef agar	....	....	....	....	....	....	....	....
Nährstoff glycerin agar	....	....	....	....	....	....	....	....

of the change in percentage of bacteria developing on the various media on the calculated efficiency is well shown.

TABLE XI.

Relation between Numbers of Bacteria and Efficiencies of Water Filters with Agar of Different Reactions.

Reaction	Relative Numbers of Bacteria			Percentage of Bacteria Removed	
	Raw Water	Filtered Water			
		A	B	A	B
−1.5	20	74	29	99.0	99.5
−1.0	90	81	44	99.7	99.8
−0.5	32	100	61	99.1	99.3
0	84	68	63	99.8	99.7
+0.6	65	88	63	99.6	99.6
+1.2	37	48	86	99.6	99.1
+1.6	100	93	100	99.7	99.6
+2.1	50	74	54	99.6	99.6
+2.6	2	6	7	99.0	98.5

TABLE XII.

Variation in Efficiency of Lawrence City Filter when Estimated by Counts of Bacteria on Agar of Different Reactions.

Sample Number	Reaction			
	−1.0	0.0	+1.0	+2.0
1	98.9	98.1	98.4	98.8
2	98.6	99.0	99.3	99.0
3	98.7	99.0	99.1	99.6
4	98.2	98.7	98.7	99.1
5	99.2	99.1	99.0	99.2
6	96.5	96.7	97.7	98.5
7	98.2	99.0	98.9	99.4
8	99.1	98.8	98.7	98.5
9	97.8	97.5	98.3	98.8
10	99.5	98.8	98.5	98.3
11	99.9	99.9	99.9	100.0
12	88.8	88.8	94.5	93.6
13	99.1	98.3	98.7	99.1
14	98.8	98.3	98.8	99.2
15	99.3	97.1	98.4	98.9
16	97.4	96.0	97.9	97.9
17	99.2	99.3	99.5	99.4
18	99.4	99.6	99.7	99.4
19	96.6	99.3	97.8	95.8
Average	98.1	97.9	98.5	98.5
Percentage of times maximum	21	5	32	42

In order to test the constancy of the determination of the efficiency on agar of different reactions, samples from the effluent of the Lawrence City filter and the applied water for that filter were plated on such media nineteen times during one month. The highest average efficiency determined during this period was 98.5, that being the same on two different reactions. The maximum variation, however, on the agar with the reaction of 1 per cent. was much less than was the variation on the other three media. The optimum reaction used for ordinary work at Lawrence is about 1.4 per cent. The results of this series of comparisons are shown in Table XII.

NOMENCLATURE OF MEDIA.

Much confusion has been caused in the past through the use of the same terms for the usual media and for special media. The recommendation of the Bacteriological Committee, that media made according to its formulæ should be specifically labeled B. C., has not been followed in a number of instances, various names being applied to media of the same composition. One example from our own experience will illustrate this: About a year ago we had occasion to repeat certain experiments of a well-known bacteriologist, and, after an investigation extending over some weeks, we found that we were unable to obtain the phenomena which he described as being both constant and characteristic. Correspondence with the original observer revealed the cause of our trouble to be in the different composition of the media used. He had stated that the base for his medium was "plain agar," meaning "standard agar, B. C.," in distinction to glycerin agar, which he used in another process; while we had used a solution of agar in water without the addition of the usual nutrient materials, this being the "plain agar" generally understood.

In a former contribution we used certain names as descriptive of our media, these names indicating in nearly every case the ingredients used in the preparation of the medium; and, furthermore, we made a distinct statement of the exact composition and method of preparation of the various media used. In the present transitional stage of bacteriological methods some such exact

expression seems necessary, that confusion may be avoided, and every attempt should be made to have such nomenclature as concise and descriptive as possible, and to have a uniform set of names for all standard and special media.

The following elementary rules are suggested as a basis for such nomenclature:

1. That liquid media in which the solvent is water be called "solutions," as, for example, "pepton solution" or "Dunham's solution," *i. e.*, pepton dissolved in water.

2. That liquid media in which the solvent is beef-infusion be called "broths," as, for example, the usual "standard broth" or "pepton broth," *i. e.*, pepton dissolved in beef-infusion.

3. That a distinction be made between media made with beef-infusion and that made with commercial beef extracts, the former being the standard ingredient, its use to be understood unless the use of the commercial extracts is distinctly stated.

4. That all media made after the formulæ of the Bacteriological Committee either have the prefix "standard," as "standard gelatin," or else be followed by the letters "B. C.," as "gelatin B. C."

5. That all media in regular use in any laboratory which approximate the "standard" or "B. C." media in composition or method of preparation, and which are substituted for the latter, be preceded by the name of the laboratory or of the individual, as "Lawrence agar," "Wurtz agar," etc.; or else be known by a name combining the various ingredients, as "pepton agar," *i. e.*, agar and pepton in water, or "plain agar," *i. e.*, agar in water.

6. That the names of special media express, as far as possible, the composition, *i. e.*, the ingredients used in their preparation.

7. That when media of standard composition are used, which vary only in reaction, this reaction should be distinctly stated, preferably in parentheses in the name, as "standard agar (+2.0)" or "agar (+2.0) B. C."

8. That when other brands of commercial pepton are used than Witte's, the same be distinctly stated, preferably by the insertion of the name of the brand in parentheses in the name, as "pepton (Merck) agar."

9. That the use of the word "nutrient" as signifying "containing pepton and beef-infusion" has become so general that it may possibly be retained without any resulting confusion, although, strictly speaking, its use is improper, since a distilled water has sufficient nutrient properties to allow of an abundant bacterial development, to say nothing of a solution of gelatin in water without the addition of beef-infusion and pepton.

CONCLUSIONS.

In standard gelatin or agar the beef broth is the most fertile source of variation, the amount of nutrient matter in beef-infusion made by the usual method varying through wide limits.

Of the two best-known commercial peptons, Merck's and Witte's, higher bacterial counts are obtained on media made with the latter.

Studies of plain agar made with various kinds of natural water show that in the majority of cases the bacteria naturally present in the given water will develop in greatest numbers in a medium made with that same water.

The salts naturally present in commercial agar have a detrimental effect, greater numbers of bacteria developing on media made with agar from which the natural salts have been washed out.

Greater numbers of bacteria will develop on media made without glycerin than on the same media containing glycerin.

With pure cultures of different kinds of bacteria on standard gelatin, agar, or Nährstoff agar, the highest counts were obtained, with the species studied, on gelatin, and the lowest on Nährstoff agar.

A reduction of the amount of Nährstoff in Nährstoff agar from 1.0 per cent. to 0.5 per cent. results in a considerable increase in the bacterial counts with various waters.

When cooked in alkaline solution, the albumoses of Nährstoff undergo certain changes in composition which render them better food material for bacteria than is the commercial product.

Studies of the efficiency of water filters in removing bacteria, when estimated by counts on different media, show that there is a considerable variation between the various media, the ratio

between the numbers of bacteria in the raw water and in the effluent being affected by the period of incubation, the reaction, and the composition of the media. With standard gelatin and Lawrence agar the ratio was less variable between different lots of agar than between different lots of gelatin.

The writers believe that it is time a uniform system of nomenclature were adopted, and that the names applied to the various media should convey definite information as to the composition, method of preparation, etc., of those media.